Individual Normal Laboratory Values

Preliminary Observations

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■ *A brief and preliminary outline is given describing a consecutive and continuing study of laboratory blood values of only 12 of 48 "normal healthy" subjects with only a few values given. The major emphasis is that of obtaining blood from each subject over a 12-week period to be repeated annually in order to determine individual values and in obtaining a chemical identification of each subject with the anticipation that more information will be available concerning the meaning and limiting parameters of "normal" biologic values. Such a study is made available through the application of modern advances in automation and the wide use of computers. It seems likely that some disorders can be discovered before clinically apparent, with the hope that consequent preventive measures and therapy may be more effective.*

The few values presented represent only a small part of those yet to be obtained. Much more work is needed in this study of normal values whose parameters must be further defined.

TO UNDERSTAND THE MEANING and application of "normal values" it is essential to discuss, however briefly, statistical terms and concepts so frequently employed in communicating results. After measuring a certain characteristic, for example the level of glucose in blood, we consider such features as the number of determinations made, the number of persons from whom the specimens were obtained, age, sex and the state of nutrition. In general we add up all the individual observations and divide by the number of determinations and obtain a value commonly called average.

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Strictly speaking, there are three values which could be obtained to represent the entire group of determinations, each of which has its own special use. These values are the mean, the mode and the median.

The mean value is what is commonly called the average value and is determined by dividing the sum of the individual observations by the number of observations, often called the sample. The mode is determined by first categorizing the values. For example, with regard to blood glucose, categories or classes of values might be established as 50 to 59 mg, 60 to 69 mg per 100 ml, and so on. One then plots the number of determinations that falls in each of these categories or classes and that class which has the largest number of values is called the mode.

The median is that value above which half of the values fall and below which half of the values fall when listed in order of magnitude. Again using blood glucose as an example, let us suppose that we have seven values, 50, 65, 80, 85, 90, 100 and 120 mg per 100 ml. The central value, 85 mg, is the median value as it divides those listed equally. In an even number of observations an average of the two middle observations would be the median. In a symmetrical continuous distribution noted in a true bell-shaped curve, the mode, median and mean are coincident; this does not apply in a skewed distribution.

Now, if we have studied a sufficiently large enough group of individuals, that is if we have an adequate sample (usually normal but not necessarily so), and if they are healthy adults in general of the same age group and sex, and we plot our individual observations in the form of a histogram or bar graph, and then fit a curve to this, we end up with what is called a normal frequency distribution or Gaussian curve. Expressed as a bell-shaped curve, the values are close to or actually bilaterally symmetrical. If our sample was adequate in all respects we would find that the mean, the mode and the median would all practically coincide. Since in further statistical applications a numerical value is needed, of the three means of expressing average, the mean is the one usually chosen to express the group.

Having obtained the mean of the set of observations made, it is useful and desirable to have some way of deciding whether or not a given value which is different from this mean is really abnormally high or abnormally low so that we can attach meaning to it. Put another way, at what point do we have to begin worrying about a value which differs from the mean? A practice more common in the past than at present was to express the mean plus the highest and the lowest observed values as the range. This method, still useful, sets unduly broad limits of range and leads to failure to detect abnormal values closer to the mean value. It is preferable to calculate limits called standard deviation (SD).

Standard deviation, by definition, is the square root of the average of the squared deviations of the individual measurements from their mean (M). Just how this mathematical expression proves useful in considering the possible significance of a single measurement may be explained as follows:

• Utilizing blood glucose measurements as an example, suppose that after measuring the blood glucose level of 100 clinically healthy people we calculated the mean was 100 mg per 100 ml and the SD was + or — 10 mg.

• Suppose that next we actually plotted on a graph the individual measurement and then drew in on the graph the position of the mean value which was 100 mg and to either side of the mean the limits of one, two and three standard deviations (SD's) which would be respectively on the positive side 110, 120 and 130 mg and on the negative side 90, 80 and 70 mg. If we then counted up the actual individual observations which fall between the mean and these successive limits of plus or minus 1, 2 and 3 SD's, we would learn something as follows: About 68 of the 100 observations would fall between minus 1 and plus 1 SD; about 95 of the 100 observations would fall in the limits set up by minus 2 and plus 2 SD's and about 99 of the 100 values would fall in the limits set up by 3 SD's to either side of the mean.

• How can we use such information to decide whether a single given value obtained subsequently on another person is within normal limits, high or low? Commonly, 2 SD's are said to represent normal limits but exactly what is meant by this? We have said that 95 of the 100 values fell within the limits of 2 SD's. Five of the values fell outside these limits. Therefore, the chances are only 5 of 100 or 1 in 20 that a value falling outside the limits of 2 SD's is normal or, putting it the other way, the chances are 19 out of 20 that such a value is abnormal.

• For these reasons, it is customary in trying to assess the significance of an individual value, as compared with the group, to set limits of 2 SD's; in some circumstances it is considered essential to be more stringent and in such instances limits of 3 SD's are set. If a value is greater than 2 SD's away from the mean, on either the high or the low side, the chances are only 1 in 20 that it is a normal value and 19 out of 20 that it is abnormal; if a given value is more than 3 SD's away from the mean, the chances are less than 1 in 100 that it is normal and conversely better than 99 in 100 that it is abnormal.

• Returning now to our example of blood glucose with a mean value of 100 mg and a SD of plus or minus 10 mg, it is possible to state that

if we receive a value of 132 mg per 100 ml, the chances are greater than 99 out of 100 that this is high; or, if we obtain a value of 66 mg/100 ml, the chances are again better than 99 out of 100 that this value is low.

• It must always be kept in mind that the original observations on which the statistical values are based must be accurate, that the method used to obtain them must be a reliable one and that the sample on which the calculations are based was sufficiently large. The greater the number of observations, the more reliable the statistical values.

• The coefficient of variation (CV) expressed in percent gives the variability or variance among the several values obtained and is determined by dividing the mean into the standard deviation, times one hundred. The closer the CV approaches zero, the more precise is the value obtained. Well defined, accurate and precise techniques are necessary and give a small "CV"—a direct guide to precision. The CV here allows value comparison between different procedures, whereas such comparison cannot be made on unit measurement (mg, gm, units and the like). Technical CV which must be distinguished from interpersonal and intrapersonal variation will not be discussed here.

Observations

Clinicians and pathologists realize that the concept of "normal values" is a distinctively changing one. The differences of normal values with age, sex and analytical technique are well known. It is customary for laboratories to establish their own specific normal values for a given healthy population in order to allow reliance on procedures performed in the laboratory, with values out of the normal range usually considered significant and possibly related to the patient's disease. It has been established by Williams,[1] and emphasized by others, that the population range of normal is greater than a group range of normal which is in turn greater than an individual range of normal. It has been shown that each individual will have his own physiologic range which may be definitely abnormal for another of the same age and sex. In order to establish specific ranges for the individual, identifying him in a concept of chemical individuality, it is necessary to perform repeated determinations in a series of tests over a period of time.

The usefulness of regular testing has been pointed out by Perry, Williams and Kupfer[2] in the testing program for physicians carried out regularly at the annual meeting of the American Medical Association. This group stated: "The repetition of selected tests year after year should permit the physician to recognize the individual's normal range. A drift away from this range should serve to warn the physician of a developing dysfunction long before the process becomes irreversible and clinical disease is manifest."

The distinction between accuracy and precision must be made. Accuracy may be defined as closeness to the true value, whereas precision applies to the agreement of repeated measurements among themselves — that is, minimal variation in results when determination is made repeatedly. A given group of values may have excellent precision but if done incorrectly there will be no accuracy. A good example is the Folin-We method of determining blood "glucose" which may be quite precise yet inaccurate.

No attempt will be made to review the many articles found in the literature on normal values.

Material and Methods

Stimulated by the work of Williams,[1] six hospitals in the United States have engaged in a Nor-

TABLE 1.—*Presented is an analysis of the mean (M), one standard deviation (SD) and coefficient of variation (CV) of a 45 year old female. Biologic values are expressed per 100 ml. Donation of one pint of blood in February 1965 produced the hemoglobin and hematocrit variances noted when compared with those of 1966 and 1967. Most values are relatively close to one another over the three-year period.*

		1965	1966	1967
Glucose mg	M	83	78	89
	1SD	8	8	5.1
	CV%	9.6	10.1	5.7
Urea Nitrogen mg	M	16	16	16
	1SD	4.3	2.8	3.2
	CV%	27	17	20
Uric Acid mg	M	4.6	5.1	5.1
	1SD	0.36	0.14	0.71
	CV%	7.8	2.7	14
Cholesterol mg	M	214	224	221
	1SD	14.6	9.7	12.9
	CV%	6.8	4.3	5.8
Oxalacetic Transaminase (Karmen Units)	M	15	11	10
	1SD	2.9	2.3	2.8
	CV%	19	21	28
Hemoglobin gm	M	12.8	13.7	13.3
	1SD	0.79	0.49	0.45
	CV%	6.1	3.6	3.4
Hematocrit mm	M	41	43	41
	1SD	5.16	1.2	1.7
	CV%	12.5	2.8	4.2

mal Laboratory Test Profile study with periodic and repetitive studies of small groups of individuals. The Sutter Community Hospitals has been engaged in this study of normal values in cooperation with Williams and his group at the National Institutes of Health. We have completed the third year of this study (1967) which includes weekly analysis of blood obtained from 12 "normal" persons who are healthy hospital employees (six men and six women, nurses, aides, technologists and porters) each week for 12 weeks, with four groups (a total of 48 individuals) studied each year.

The hospital employees were chosen by personal interview after evaluating their completed detailed questionnaires and information from their private physicians concerning history and physical findings. Excluded were those with any persistent symptom or evidence of cardiopulmonary, hepatic, renal or other systemic disease. No attempt was made to alter the individual and usual diet. Annual reviews of health and physical condition were made before starting a 12-week period of testing. Information was exchanged with the private physician and correlated with the values obtained. All specimens were obtained in a fasting state after arrival of the subject at the hospital for the regular work period. The few personnel on medication were those taking estrogens, occasional aspirin and one on colchicine (note Table 2, uric acid). At present the first group is being studied for the fourth year in a row, with over 52,000 tests completed by the end of 1967. The study is planned to extend for five years. It is quite evident that a vast amount of data will become available after computer statistical analysis.

TABLE 2.—*The mean (M), standard deviation (SD) and coefficient of variation (CV) are given in a 56 year old male over a three-year period, as a result of 12 weekly similar determinations each year. Biologic values are expressed per 100 ml. Here also most values are close to one another from year to year. Note the high uric acid which was not associated with symptoms of gout until September of 1967.*

		1965	*1966*	*1967*
Glucose	M	86	82	93
mg	1SD	5	6.2	9.3
	CV%	5.8	7.5	10
Urea Nitrogen	M	16	15	16
mg	1SD	2.1	2.2	1.2
	CV%	13	15	7.5
Uric Acid	M	9.7	8.8	10.1
mg	1SD	0.46	0.22	1.04
	CV%	4.7	2.2	10.3
Cholesterol	M	247	240	248
mg	1SD	7.0	10.7	10.9
	CV%	2.8	4.5	4.4
Oxalacetic	M	29	20	25
Transaminase	1SD	3.9	2.7	4.4
(Karmen Units)	CV%	13	13.5	17.5
Hemoglobin	M	13.9	14.0	14.7
gm	1SD	0.38	0.42	0.95
	CV%	2.7	3.0	6.5
Hematocrit	M	45	44	45
mm	1SD	1.0	1.4	1.7
	CV%	2.2	3.2	3.8

Automated equipment consisted of five double channeled AutoAnalyzers for the chemistry procedures, Coulter counters for the erythrocyte and leukocyte counts and a Beckman Analytrol for paper electrophoresis. Such equipment allows, with careful quality control, a relatively narrow range of reproducibility and precision. As in any good quality control program, such tests can be performed accurately with adequate controls and standards. The tests performed on these 48 persons at Sutter Community Hospitals consist of blood glucose, blood urea nitrogen, uric acid, cholesterol, glutamic oxalacetic transaminase (Karmen units), alkaline phosphatase (Babson-Reed units), total protein, albumin and globulin, calcium, magnesium, serum protein electrophoresis with evaluation of alpha, beta and gamma globulins, as well as albumin, total white count, hematocrit, hemoglobin, red cell count and differential. All of the values were done in duplicate with tabulation of all values obtained in arriving at mean and standard deviation.

Information obtained in the study is being processed by computers at the National Institutes of Health with anticipation that much information will become available during the five years of study. The values given in the figures and tables below were obtained not by these computers but by hand electrical calculators.

Expected data to be obtained may answer the following questions: Is there a relationship between single or several values and age, sex or the development of disease? Is there possible variance with season, with geography (in relationship to other cooperative groups in the United States)? Why do some persons have a narrower variance of a given value then do others? Why do some persons have a narrower interpersonal variance in one year than in another? Will changes in some blood values be of greater help in predicting subclinical or overt disease?

The observations presented in this brief paper are only a few of those which apply to 12 individuals of the first group obtained over 12 successive

weeks each year in January, February and March of 1965, 1966 and 1967.

Results

Chart 1 is a bar-graph presentation of uric acid values obtained (mean and one standard deviation) from 12 individuals. Note that one man 59 years of age (M 59) was available only in 1965 and 1966 (died in October 1967) and that M 35 was available only in 1965 for this study. Note that each individual maintains a close mean value and one standard deviation over a three-year period, quite characteristic of most values obtained. The individual M 56 in Chart 1 was without any symptoms of gout until September of 1967. This feature suggests that a persistent elevation of serum uric acid should alert the clinician to the possibility of gout developing in his patient.

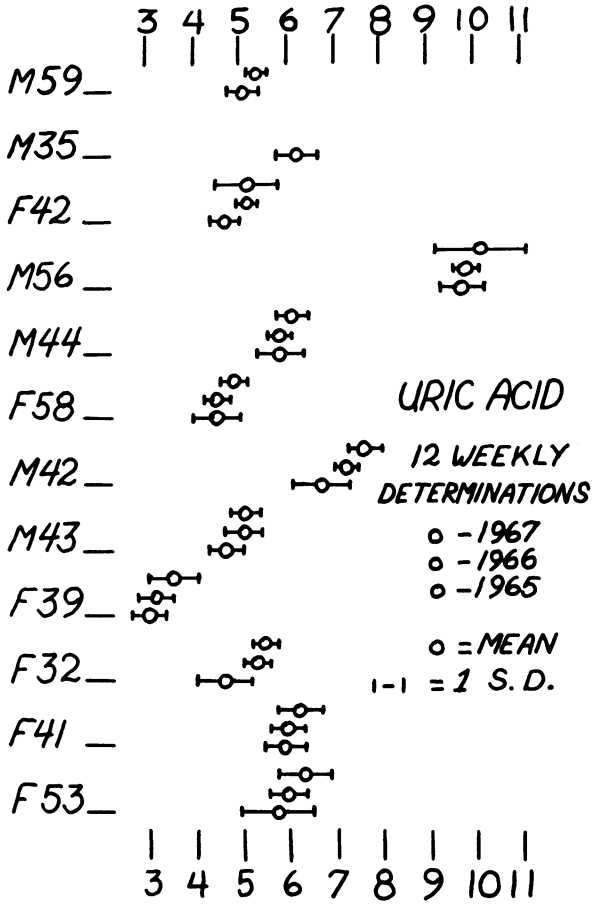

Chart 1.—Uric acid values are given stating the mean and one standard deviation of 12 successive weekly determinations each year over a three-year period. In groups of 3 the values for each subject represent from bottom to top the years 1965, 1966 and 1967. The left column identifies each subject by sex and age. Subjects M59 and M35 did not complete the three-year study. Values are given in mg per 100 ml.

Those individuals in our study with mild obesity had uniformly elevated values for blood glucose and cholesterol.

Chart 1 shows a "clustering" of uric acid values which may have some practical value if in subsequent years such values for one subject shift beyond two or three standard deviations. We may ask why does one subject maintain a narrow range (SD) and others do not? This feature was more evident in some of the other values obtained, not illustrated here. Would this suggest a tight and more restricted metabolism of uric acid, urea, glucose and the like?

In Chart 2 we see the mean values and standard deviations of 11 blood substances examined from a woman 42 years of age with a group mean of 1965 for comparison. The values of each of the other 11 persons in this group can be outlined in a similar manner, with each having a distinctive and non-identical pattern.

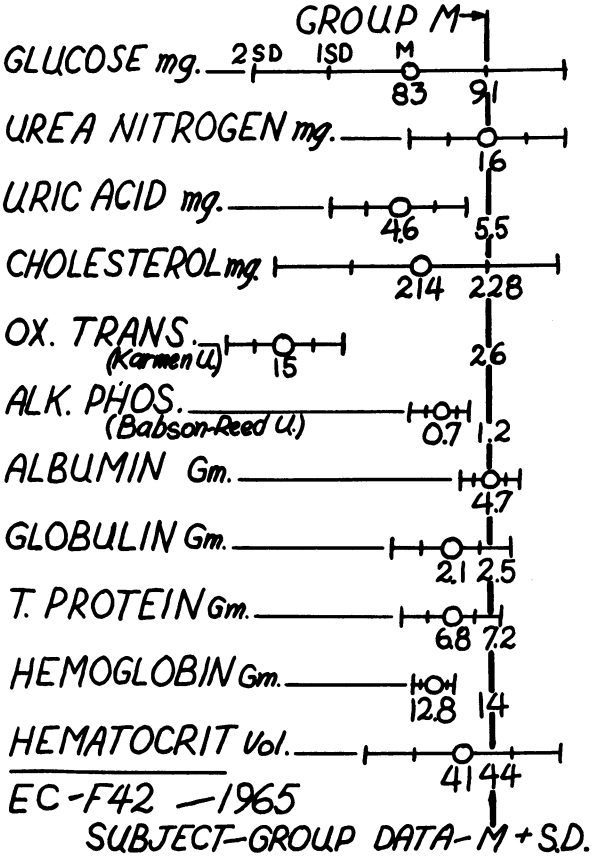

Chart 2.—Eleven substances are listed comparing the subject and group mean of 12 weekly determinations in 1965 and listing one and two standard deviations of each substance. Each subject thus graphically tabulated was unique from the 11 others in the same group. Biologic values are given in units per 100 ml.

Each bar-graph is placed in relative position to the heavy vertical line which acts as the group mean. Not indicated is the group SD which, as expected, is wider than the individual SD.

Recently, Williams,[4] in describing the effects of laboratory automation in clinical medicine, presented in chart form a hypothetical example of an individual's biologic chemistry profile. This chart gives base-line values which can be used in comparison with subsequent changes of specific tests. In a similar manner our data on 48 individuals can be used as a base-line for future reference.

In Tables 1, 2 and 3 we find for seven blood values the M, 1 SD and CV given for three individuals in the first group each over a three-year period. The patient in Table 1 can be seen to have a wide SD and CV in 1965 for hematocrit (less for hemoglobin) because she donated a pint of blood in February. It may be pointed out that the CV presented in each individual is illustrative of physiologic or biologic variance, which of necessity must include technical variance. Other figures and charts thus prepared graphically illustrate and further emphasize the concept of individual chemical or hematologic characteristics.

In Table 4 are found nine values obtained from pooled serum in 1965 over an 11-week period representing only a part of our quality control program.

The stated physiologic variance for the first five values given in Tables 1, 2 and 3 for 1965 must be compared with similar values in Table 4 which reflect the technical variance of identical tests. For example, the glucose variance of 1.6 percent in Table 4 when subtracted from the glucose variance of 9.6 percent in Table 1 will give us a true physiological variance of 8 percent. The other four values can be similarly evaluated. The SD and CV for the first five quality control values noted in Table 4 for 1965 can be seen to be less than those for the 1965 values depicted in Tables 1, 2 and 3, except for the cholesterol values noted in Table 2.

Normal values as observed at Sutter Community Hospitals are shown in Table 5.

TABLE 3.—*Values similar to those noted in Table 1 and 2, a 46 year old male. Biologic values are expressed per 100 ml. Relatively narrow ranges of values are maintained over a three-year period.*

		1965	1966	1967
Glucose mg	M	85	80	85
	1SD	9	4.4	4.0
	CV%	10	5.5	4.7
Urea Nitrogen mg	M	17	16	17
	1SD	1.7	2.2	2.0
	CV%	10	14	11.8
Uric Acid mg	M	5.8	5.8	6.1
	1SD	0.53	0.08	0.34
	CV%	9.1	1.3	5.5
Cholesterol mg	M	198	198	205
	1SD	14.3	13.7	9.73
	CV%	7.2	6.9	4.7
Oxalacetic Transaminase (Karmen Units)	M	27	14	21
	1SD	3.5	3.3	2.43
	CV%	13	23	11.5
Hemoglobin gm	M	15.3	15.0	15.4
	1SD	0.11	0.49	0.45
	CV%	0.7	3.2	2.9
Hematocrit mm	M	48	47	47
	1SD	1.28	3.8	1.08
	CV%	2.7	8.1	2.3

TABLE 4.—*Quality control values are listed as a result of determinations performed on a pooled serum from 11 weekly determinations in January-February-March 1965, showing the mean (M), one and two standard deviations (SD) and coefficient of variation (CV). The CV as a variance can be noted to be much narrower than the physiological variations listed in other tables. A complete quality control program is a most essential part of evaluating the precision and accuracy of a laboratory. Values are expressed in the usual mg, gm and units per 100 ml.*

	M	1 SD	2 SD	CV
Glucose	61	1.0	2.0	1.6%
Urea Nitrogen	16	0.31	0.6	1.9%
Uric Acid	3.1	0.07	0.14	2.2%
Cholesterol	128	8.2	16.4	6.4%
Glutamic Oxalacetic Transaminase (Karmen Units)	18	2.0	4.0	11.2%
Alkaline Phosphatase (Babson-Reed Units)	0.9	0.24	0.5	27.0%
Albumin	2.9	0.09	0.18	3.1%
Globulin	1.3	0.09	0.18	6.9%
Total Protein	4.1	0.11	0.2	2.7%

TABLE 5.—*Normal Values as Observed in Sutter Community Hospitals*

	Range per 100 ml
Glucose	75-110 mg
Urea Nitrogen	6-23 mg
Uric Acid	3-6 mg
Cholesterol	150-250 mg
Oxalacetic Transaminase (Karmen Units)	up to 40 Karmen Units
Alkaline Phosphatase (Babson-Reed Units)	1.5-4 Babson-Reed Units
Albumin	4.5-5.5 gm
Globulin	1.5-3.0 gm
Total Protein	6-8 gm
Hemoglobin	M 14-18 gm F 12-16 gm
Hematocrit, percent	M 42-52 F 37-47

Procedures those of routine Technicon N methods. Hemoglobin by cyanmethemoglobin method.

Discussion

The values given above in figures and tables actually form only a small amount of the data that are to be obtained. Several points may be emphasized.

- Each individual has a specific and distinctive pattern of biologic values.
- It is probable that no two individuals are identical in this regard.
- Individual (intrapersonal) mean, standard deviation and coefficient of variation have narrower ranges than similar (interpersonal) group values.
- These group values are again narrower than population values.
- Much has yet to be learned about the parameters of "normal" laboratory values.

It is anticipated that the survey will continue for several more years in order to study possible variations with age, sex, ethnic background and time in specific individuals. It is also likely that some variation in the method of study will be made for subsequent comparison with previous values obtained. This variation in time over the years may present us with much information not yet available. This study would not have been possible without the use of automated equipment which has the value of performing a large number of tests in a uniform manner at a reasonable cost. The potential of such automation has not yet been reached and with the addition of computers the retrieval of information is infinitely greater.

Williams showed that many tests repeated on individuals over long periods may reveal a similar constancy compared with the wider range for a healthy population. The results of our test patterns noted above have confirmed the relative individuality of these test patterns and indicate the desirability of expanding the number of procedures and involving a larger number of people. For uniformity and comparability of results from one time to another on the same person and for comparison of individuals, continuous quality control and a careful evaluation of the performance limits of each test are necessary. This must of necessity apply to automated procedures.

It is possible that such a study might allow us to predict the development of various disorders before clinically apparent. One might expect that "pre-diabetes" might be more easily detected. One of many blood urea nitrogen determinations may be abnormal enough to warrant further evaluation of possible renal disease. Serial determinations of serum alkaline phosphatase may reveal early hepatic disease. We are all aware of the changes of the serum glutamic oxalacetic transaminase and lactic dehydrogenase as myocardial infarction develops and then progresses. Determining a newborn's blood phenylaline level will allow the detection of phenylketonuria (PKU) before clinical evidence of disease. Many such additional examples may be given. A study such as applied above must be done with the realization that inherent problems are present, several of which are:

- It is essential that blood be obtained from completely "healthy" persons ("healthy" to be defined).
- The technique must be well established and accurate with good precision.
- An adequate quality control program must be a part of such a study.
- The chemical values of many tests will vary with the method and instruments being used.
- The time that the blood (urine, spinal fluid, etc.) is withdrawn may produce variables due to diet, drugs, diurnal variations, etc.
- Some medications will interfere with certain test values.
- Further work is necessary to improve precision, accuracy and quality control in medical laboratories.

At a recent conference[3] on "Normal Laboratory Test Profiles," held at the National Institutes of Health, other laboratories and hospital groups are now cooperating in this endeavor with the expectation that more accumulated data will be obtained to be used as a guide in further defining normal values.

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